EXHIBIT C

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Purpose - To label synthetic probe ChtA176 with ^{32}P by Kinasing for Chlamydia assay development.

Reagents -

Synthetic probe ChtA176 mm 320-39 1.28 OD/ml 64 µg/ml
10X Kinase buffer, from RB + mm, aliquot obtained (391:18)
T4 Kinase, BRL, 10 u/µl Lot 62111
γ-^{32}P-ATP 3000 Ci/mmol #G551, rcd. 10 µCi/µl
Teflon, acid-base treated, from Mark H.
5 m NaCl DEB NB 151:48
1% SDS diluted from 20% (DK)
1 M Tris, pH 8.2 (DK)
100 mM EE L/N 500.38
4 M LiCl from DK
Proteinase K 20 mg/ml 333:11
Glycogen HL-1 ~40 mg/ml in 10% EtOH (DK)
Phenol from DK ( )
Chloroform
10% TCA
BSA, 10 mg/ml from BRL Lot# 4046

Procedure

1. Follow same procedure used (391:18-20)
Kinase reaction included 1 µl of ChtA176 (64 ng)

Results

Column rinses in 0.3 m NaCl and H_2O:
$\equiv (9.6 \times 10^4)\left(\frac{3300}{5}\right) = 6.3 \times 10^7$ cpm

Straight count - $(6.2 \times 10^4)\left(\frac{200}{1}\right)\left(\frac{50}{10}\right) = 6.2 \times 10^7$ cpm } Why so low?

Input dpm = 2.2×10^8 dpm

EtOH super - $(1.1 \times 10^3)\left(\frac{2400}{5}\right) = 5.3 \times 10^5$ cpm

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Results, cont.

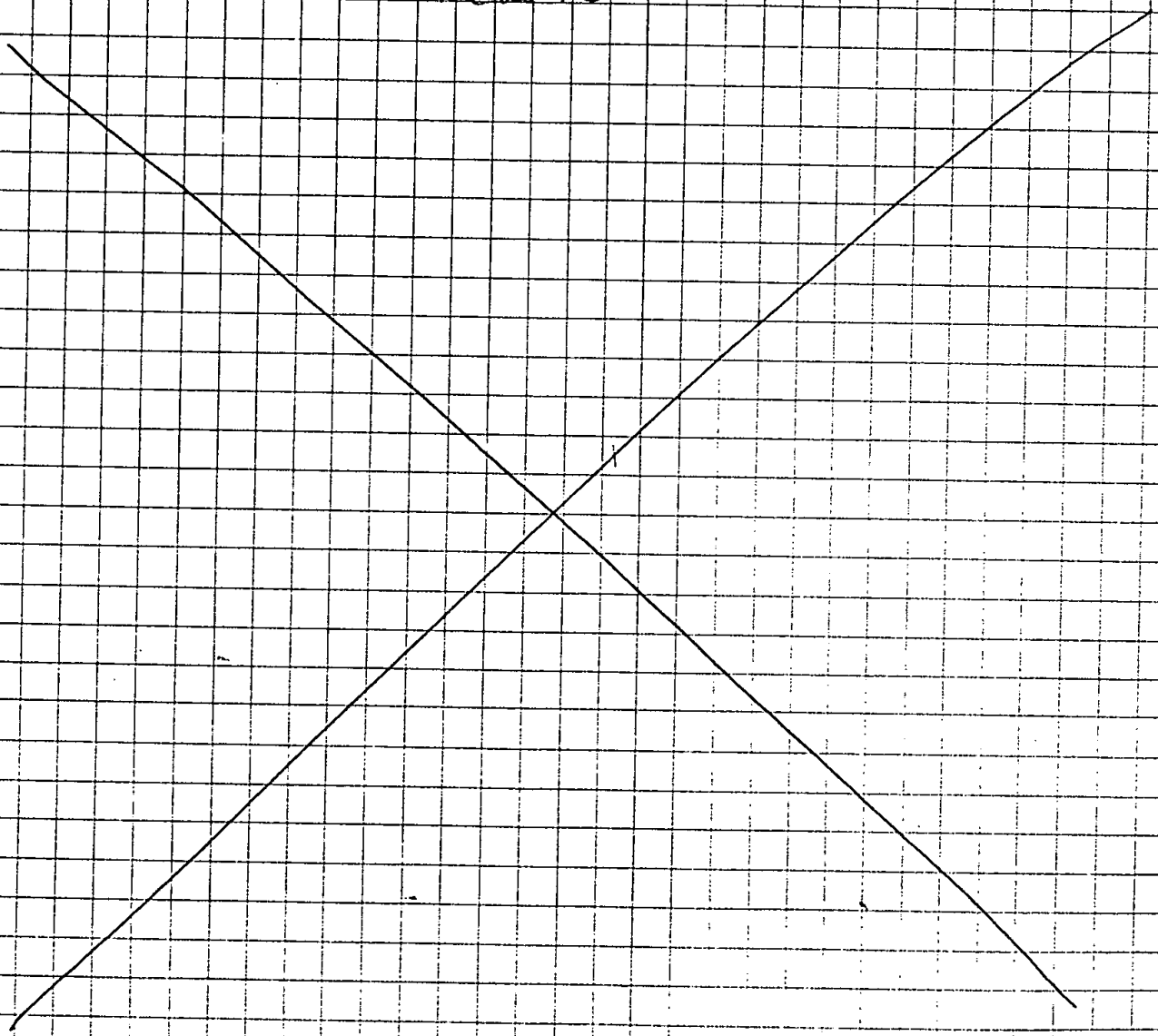
TCA ppt able cpm = $(1.2\times10^4)\left(\frac{200}{1}\right)\left(\frac{50}{10}\right) = 1.2\times10^7$ cpm in 64 ng

specific act. = 1.9×10^8 cpm/μg

Final cpm in 100 μl H_2O $(1.8\times10^5)\left(\frac{100}{2}\right) = 9\times10^6$ cpm 9×10^4 cpm/μl 75% recovery

diluted total sample to 300 μl 0.02% SDS → 3×10^4 cpm/μl final

store at 4°C



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